UNIVERSITI PUTRA MALAYSIA

GENETIC POTENTIAL OF SELECTED SWEET CORN INBRED LINES AND ANALYSIS OF THEIR COMBINING ABILITY ASSISTED BY MICROSATELLITE DNA MARKERS

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By

PEDRAM KASHIANI

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

May 2012

Dedicated With Love

To :

My Kind Father, Ahmad

&

My Beloved Mother, Fatimah

For Their Endless Love, Support and Sacrifices

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

GENETIC POTENTIAL OF SELECTED SWEET CORN INBRED LINES AND ANALYSIS OF THEIR COMBINING ABILITY ASSISTED BY MICROSATELLITE DNA MARKERS

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May 2012

Chairman: Prof. Ghizan Saleh, PhD

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A study was conducted to evaluate the potential of sweet corn inbred lines developed from different source populations, to determine genetic diversity among those inbred lines based on agronomic performance and microsatellite DNA markers, to identify major heterotic groups among the inbred lines for selection of potential parents for hybrid production, and to evaluate combining abilities among selected parental inbred lines in a diallel cross. Performance and heterosis of the single-cross hybrids were evaluated at two different locations, Serdang and Sepang. Genetic distances among the inbred lines revealed by the microsatellite markers were used to predict hybrid performance.

Thirteen selected sweet corn inbred lines adapted to the tropical environment were evaluated for their performance. Significant variations in agronomic performance were revealed by the inbred lines evaluated, indicating the presence of high genetic diversity

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among them for potential utilization towards production of hybrid varieties. Among the inbred lines, the highest husked fresh ear yield was obtained from TSN-S8 with value of 6549 kg ha^{-1}, but this was not significantly higher than those obtained from TSK-S8, BAK-S8 and MAN-S8 (6483, 6402 and 5798 kg ha^{-1}, respectively).

Microsatellite markers exhibited high Shannon's information index (I), Nei's expected heterozygosity (Nei's), and polymorphic information content (PIC), with mean values of 1.053, 0.586 and 0.582 respectively, indicating their appropriateness in detecting genetic variability among the inbred lines. Molecular analysis of variance showed that 92.88% of the total variation occurred among the inbred lines while only 7.11% occurred within the inbred lines. Shannon's information index and Nei's gene diversity coefficient showed that Chromosome 10 was the most informative chromosome (I = 1.311 and Nei's = 0.703), while Chromosome 2 had the least variation among the chromosomes evaluated (I = 0.762 and Nei's = 0.456). Chromosome 4 was found to possess the highest number of pairs of alleles in linkage disequilibrium (122 pairs), while Chromosome 6 was found to have only six pairs of alleles in linkage disequilibrium. The inbred lines were assigned into five main heterotic groups based on their agronomic and molecular characteristics. MAS-S8, DFS-S8, TSN-S8, TZF-S8 and SBY-S8 each from a different heterotic group were selected as parental inbred lines for a diallel cross.

From results of evaluations on the hybrids, Hybrids H2 and H1 were found to have the highest fresh ear yield in Serdang, with mean values of 15475 and 15124 kg ha^{-1}, respectively, while Hybrids H3 and H1 revealed the highest fresh ear yield in Sepang (15143 and 14998 kg ha^{-1}, respectively). There was no significant difference in fresh ear

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yields between these hybrids and that of the control variety (Hybrid 968), indicating that fresh ear yield of these hybrids could be comparable to those of the commercial hybrids available in the market. Among the traits measured, husked fresh ear yield exhibited the highest mid- and high-parent heterosis at the two locations of study. In general, Hybrids H1, H2 and H3 had high and consistent estimates of mid- and high-parent heterosis for all traits measured, indicating that they accumulated favorable genes possessed by their parental inbred lines.

Combining ability analysis revealed presence of significant additive and non-additive gene actions in control of the traits measured. However, additive gene effects were found to be relatively more important in the control of yield and yield components in the hybrids, based on greater GCA mean squares than SCA mean squares that they possessed. DFS-S8, with the highest positive and significant GCA for husked fresh ear yield in Serdang, Sepang and the two locations combined (3091.54, 2370.98 and 2731.26 respectively), was identified as the best general combiner among the parental inbred lines for high yield and yield components in its crosses. DFS-S8 X MAS-S8 (H3), DFS-S8 X SBY-S8 (H4), TFZ-S8 X TSN-S8 (H5) and TFZ-S8 X SBY-S8 (H7) were the best cross combinations, with favorable positive SCA estimates for yield and yield related traits at each location and locations combined. The Wr-Vr graphical analysis showed that non-additive gene effects that controlled the traits were overdominance for all traits measured. The overdominance mode of gene action was also revealed through greater estimates of variation due to dominance effects of genes

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(H_1 and H_2) than additive effect (D), and estimates of average degree of dominance higher than unity.

The high true-sense heritability estimates (more than 50%) revealed the presence of high proportion of genetic variability in the whole phenotypic variability, while the presence of high broad-sense heritability estimates (more than 80%) indicate a close correspondence between the genotype and the phenotype, due to a relatively smaller contribution of the environment to the phenotype. The narrow-sense heritability estimates revealed were however generally lower than the broad-sense heritability estimates, indicating the preponderance of non-additive gene actions in the inheritance of all traits measured.

Significant positive associations were obtained between genetic similarities of the parental inbred lines estimated based on microsatellite markers with more than 30% polymorphic bands and SCA estimates for number of ears per hectare, husked and dehusked ear weights, TSS, husked and dehusked ear diameter and number of kernels per row. This indicates the effectiveness of molecular markers for prediction of hybrid performance from those tropical sweet corn inbred lines. In conclusion, among the 10 tropical hybrids produced, Hybrids H1, H2 and H3 exhibited high yield performance and high magnitude of yield components at each location and the two locations combined. It is thus suggested that these promising hybrids be further tested in large scale multi-locational trials before their release as new tropical sweet corn hybrid varieties in Malaysia.

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Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

POTENSI GENETIK TITISAN INBRED JAGUNG MANIS TERPILIH DAN ANALISIS KEUPAYAAN BERGABUNGNYA DIBANTU OLEH PENANDA MIKROSATELIT DNA

Oleh

PEDRAM KASHIANI

Mei 2012

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Satu kajian telah dijalankan untuk menilai potensi titisan-titisan inbred jagung manis yang dibentuk daripada sumber populasi yang berbeza, untuk menentukan kepelbagaian genetik di kalangan titisan-titisan inbred berkenaan berdasarkan prestasi agronomi dan penanda DNA mikrosatelit, untuk mengenalpasti kumpulan-kumpulan heterotik utama di kalangan titisan-titisan inbred tersebut untuk pemilihan induk yang berpotensi bagi pengeluaran hibrid, dan untuk menilai keupayaan bergabung di kalangan induk titisan inbred terpilih dalam kacukan diallel. Prestasi dan heterosis hibrid-hibrid kacukan tunggal dinilai di dua lokasi yang berbeza, Serdang dan Sepang. Jarak genetik di kalangan titisan-titisan inbred yang ditunjukkan oleh penanda mikrosatelit digunakan untuk meramalkan prestasi hibrid.

Tiga belas titisan inbred jagung manis yang telah beradaptasi di persekitaran tropika telah dinilai untuk prestasinya. Variasi yang ketara dalam prestasi agronomi telah

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ditunjukkan oleh titisan-titisan inbred yang dinilai, menunjukkan terdapatnya kepelbagaian genetik yang tinggi di kalangannya untuk potensi penggunaan ke arah pengeluaran varieti hibrid. Di kalangan titisan-titisan inbred, hasil tongkol segar berkulit tertinggi diperolehi dari TSN-S8 dengan nilai 6549 kg ha^{-1}, tetapi ianya secara berertinya tidak lebih tinggi daripada yang diperoleh dari TSK-S8, BAK-S8 dan MAN-S8 (masing-masing 6483, 6402 dan 5798 kg ha^{-1}).

Penanda mikrosatelit menunjukkan nilai yang tinggi bagi maklumat indeks Shannon (I), heterozigositi dijangka Nei (Nei's), dan kandungan maklumat polimorfik (PIC) dengan nilai min masing-masing sebanyak 1.053, 0.586 dan 0.582, menunjukkan kesesuaiannya dalam mengesan variabiliti genetik di kalangan titisan-titisan inbred tersebut. Analisis varians molekul menunjukkan bahawa 92.88% daripada jumlah variasi berlaku di kalangan titisan inbred manakala hanya 7.11% berlaku dalam titisan inbred. Indeks maklumat Shannon, dan pekali kepelbagaian gen Nei menunjukkan bahawa Kromosom 10 adalah kromosom yang paling bermaklumat (I =1.311 dan Nei's = 0.703), manakala Kromosom 2 mempunyai variasi yang paling rendah di kalangan kromosom yang dinilai (I = 0.762 and Nei's = 0.456). Kromosom 4 didapati mengandungi bilangan pasangan alel yang tertinggi dalam ketakseimbangan rangkaian (122 pasang), manakala Kromosom 6 didapati mengandungi hanya enam pasangan allel dalam ketakseimbangan rangkaian. Titisan inbred dibahagikan kepada lima kumpulan heterotik utama berdasarkan kepada ciri-ciri agronomi dan molekul. MAS-S8, DFS-S8, TSN-S8, TZF-S8 dan SBY-S8, tiap satunya dari kumpulan heterotik yang berbeza telah dipilih sebagai induk titisan inbred untuk kacukan diallel.

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Daripada keputusan penilaian hibrid, Hibrid H2 dan H1 didapati mempunyai hasil tongkol segar yang tertinggi di Serdang dengan nilai min sebanyak masing-masing 15475 dan 15124 kg ha^{-1}, manakala hibrid H3 dan H1 didapati mempunyai hasil tongkol segar tertinggi di Sepang (masing-masing 15143 dan 14998 kg ha^{-1}) di Sepang. Tidak terdapat perbezaan yang bererti dalam hasil tongkol segar antara hibrid-hibrid tersebut dan varieti kawalan (Hybrid 968), menunjukkan bahawa hasil tongkol segar hibrid tersebut setanding dengan hasil hibrid komersial yang terdapat dalam pasaran. Antara sifat-sifat yang diukur, hasil tongkol segar berkulit mempamerkan heterosis pertengahan induk dan induk terbaik yang tertinggi di dua lokasi kajian. Secara amnya, Hibrid H1, H2 dan H3 memperolehi anggaran heterosis pertengahan induk dan induk terbaik yang tinggi dan konsisten untuk semua sifat-sifat yang diukur, menunjukkan bahawa ianya telah menghimpunkan gen-gen yang diingini yang dimiliki oleh titisan-titisan induk inbrednya sebagai.

Analisis keupayaan bergabung menunjukkan terdapatnya tindakan gen menambah dan tidak menambah yang bererti dalam pengawalan ciri-ciri yang diukur. Walau bagaimanapun, kesan gen menambah didapati secara perbandingannya, lebih penting dalam pengawalan hasil dan komponen-komponen hasil di dalam hibrid-hibrid, berdasarkan varians GCA yang lebih besar daripada varians SCA yang dimilikinya. DFS-S8 dengan GCA positif dan bererti yang tertinggi untuk hasil tongkol segar berkulit di Serdang, Sepang dan apabila kedua-dua lokasi digabungkan (masing-masing 3091.54, 2370.98 dan 2731.26) telah dikenalpasti sebagai penggabung am yang terbaik di kalangan induk titisan inbred bagi hasil dan komponen-komponen hasil yang tinggi

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dalam kacukannya. DFS-S8 × MAS-S8 (H3), DFS-S8 × SBY-S8 (H4), TFZ-S8 × TSN-S8 (H5) dan TFZ-S8 × SBY-S8 (H7) adalah kombinasi kacukan terbaik, dengan anggaran SCA positif yang diingini bagi hasil dan ciri-ciri yang berkaitan hasil di setiap lokasi dan apabila kedua-dua lokasi digabungkan. Analisa grafik Wr-Vr menunjukkan bahawa kesan gen bukan menambah yang mengawal ciri-ciri adalah kesan dominan melampau untuk kesemua ciri-ciri yang diukur. Mod tindakan gen dominan melampau juga ditunjukkan melalui anggaran variasi yang lebih besar yang diakibatkan oleh kesan gen dominan (H1 dan H2) berbanding kesan gen menambah (D), dan anggaran purata darjah dominan yang lebih tinggi daripada satu.

Anggaran kebolehwarisan sebenar yang tinggi (lebih daripada 50%) menunjukkan wujudnya sebahagian besar daripada variabiliti genetik pada keseluruhan variabiliti fenotip, manakala kewujudan anggaran kebolehwarisan luas yang tinggi (lebih daripada 80%) menunjukkan perkaitan yang rapat antara genotip dan fenotip, disebabkan oleh sumbangan persekitaran yang agak kecil kepada fenotip. Anggaran kebolehwarisan sempit yang dipamerkan bagaimanapun pada umumnya lebih rendah daripada anggaran kebolehwarisan luas, menunjukkan kewujudan tindakan gen bukan menambah yang tinggi dalam pewarisan semua ciri-ciri yang diukur.

Perkaitan yang positif secara bererti telah diperolehi antara persamaan genetik induk titisan inbred yang dianggarkan berdasarkan penanda mikrosatelit dengan lebih daripada 30% jalur polimorfik dan anggaran SCA untuk bilangan tongkol sehektar, berat tongkol berkulit dan tanpa kulit, jumlah kepekatan pepejal terlarut (TSS), diameter tongkol

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berkulit dan tanpa kulit dan bilangan bijian sebaris. Ini menunjukkan keberkesanan penanda molekul bagi menjangka prestasi hibrid dari titisan inbred jagung manis tropika tersebut. Kesimpulannya, antara 10 kacukan hibrid-hibrid tropika yang telah dihasilkan, Hibrid H1, H2 dan H3 mempamerkan prestasi hasil yang tinggi dan magnitud komponen-komponen hasil yang tinggi di setiap lokasi dan gabungan antara kedua-dua lokasi. Oleh itu adalah dicadangkan bahawa hibrid-hibrid yang berpotensi ini diuji selanjutnya di dalam pengujian pelbagai lokasi yang berskala besar sebelum diisytiharkan sebagai varieti hibrid jagung manis tropika yang baharu di Malaysia.

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I certify that a Thesis Examination Committee has met on 18 May 2012 to conduct the final examination of Pedram Kashiani on his thesis entitled "Genetic Potential of Selected Sweet Corn Inbred Lines and Analysis of Their Combining Ability Assisted by Microsatellite DNA Markers" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

_____

PEDRAM KASHIANI

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TABLE OF CONTENTS

		Page
DEDICATION		**ii**
ABSTRACT		**iii**
ABSTRAK		**vii**
ACKNOWLEDGEMENTS		**xii**
APPROVAL		**xv**
DECLARATION		**xvii**
LIST OF TABLES		**xxii**
LIST OF FIGURES		**xxvi**
LIST OF ABBREVIATIONS		**xxviii**
CHAPTER		
I	**INTRODUCTION**	**1**
II	**LITERATURE REVIEW**	**4**
2.1	Corn	4
2.2	Origin and Domestication of Sweet Corn	6
2.3	Morphology and Botany of Sweet Corn	7
2.4	Sweet Corn in Malaysia	8
2.5	Sweet Corn Inbred Lines	10
2.6	Heritability	11
2.7	Correlations among Traits	13
2.7.1	Phenotypic Correlations	14
2.7.2	Genetic Correlations	16
2.8	Heterosis	17
2.9	Combining Ability Analysis and Gene Action	22
2.10	Relationship between Heterosis and Combining Ability	25
2.11	Molecular Markers for Diversity Analysis	27
2.12	Genetic Diversity in Sweet Corn	29
2.13	Statistical Analysis for Molecular Diversity Data	34
III	**DEVELOPMENT AND PERFORMANCE OF TROPICAL SWEET CORN INBRED LINES**	**39**
3.1	Introduction	39
3.2	Materials and Methods	41
3.2.1	Variation and Genetics of Selected Inbred Lines	41
3.2.1.1	Location of Experiment	41
3.2.1.2	Plant Materials	41
3.2.1.3	Experimental Design and Plot Arrangement	42
3.2.1.4	Cultivation Practices	43
3.2.1.5	Data Collection	43

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3.2.2 Data Analyses 45
3.2.2.1 Performance of Inbred Lines 45
3.2.2.2 Broad-sense Heritability of Traits 46
3.2.2.3 Pearson's Correlation Estimates among Traits 47
3.2.2.4 Visualization of Relationship between Inbred Lines and Traits 49
3.3 Results 50
3.3.1 Performance of Inbred Lines 50
3.3.2 Broad-sense Heritability for Traits Measured on Inbred Lines 57
3.3.3 Correlations among Traits 58
3.3.3.1 Phenotypic Correlations 58
3.3.3.2 Genetic Correlations 62
3.3.4 Association of Traits with Inbred Lines 65
3.4 Discussion 68

IV GENETIC VARIATION AMONG SWEET CORN INBRED LINES REVEALED BY MICROSATELLITE DNA MARKERS 74

4.1 Introduction 74
4.2 Materials and Methods 78
4.2.1 Plant Materials 78
4.2.2 DNA Extraction 78
4.2.3 DNA Quantification 80
4.2.4 Selection of Microsatellite Primers 80
4.2.5 DNA Amplification using Polymerase Chain Reaction (PCR) 82
4.2.6 Metaphore Agarose Gel Electrophoresis 82
4.2.7 Statistical Analysis 83
4.3 Results 89
4.3.1 Microsatellite Primer Efficiency in Detecting Genetic Variation among 13 Tropical Sweet Corn Inbred Lines 89
4.3.2 Genetic Differentiation among 13 Tropical Inbred Lines Revealed by Microsatellite DNA Markers 96
4.3.3 Demarcation of Informative Chromosomes in 13 Tropical Sweet Corn Inbred Lines Using Microsatellite DNA Markers 104
4.4 Discussion 110

V SELECTION OF PARENTAL LINES FOR HYBRID PRODUCTION BASED ON PHENOTYPIC AND MOLECULAR GENETIC DIVERSITIES 121

5.1 Introduction 121
5.2 Materials and Methods 127
5.2.1 Data Collection 127
5.2.2 Statistical Analyses 128
5.3 Results 131

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5.3.1 Genetic Distance among Sweet Corn Inbred Lines based on Phenotypic Characteristics 131
5.3.2 Genetic Distances among Sweet Corn Inbred Lines based on Microsatellite DNA Markers 138
5.3.3 Selection of Parental Lines for Hybrid Development Based on Phenotypic and Molecular Characteristics 146
5.4 Discussion 151

VI PERFORMANCE AND HETEROSIS OF SINGLE CROSS SWEET CORN HYBRIDS, AND COMBINING ABILITY OF THEIR PARENTAL INBRED LINES 157

6.1 Introduction 157
6.2 Materials and Methods 161
6.2.1 Plant Materials 161
6.2.2 Establishment of crosses 162
6.2.2.1 Preparation of Ear-shoots 162
6.2.2.2 Preparation of Tassels 162
6.2.2.3 Hand Pollinations 163
6.2.3 Field Evaluations 163
6.2.4 Statistical Analyses 164
6.2.4.1 Performance of Hybrids 164
6.2.4.2 Heterosis 165
6.2.4.3 Diallel Analysis by Hayman's Graphical Method 166
6.2.4.4 Diallel Analysis by Griffing's Method 167
6.2.4.5 Genetic Components of Variation and Heritability Estimates 170
6.2.4.6 Relationship between Molecular Genetic Similarities of Parental Lines and Hybrid Performance 173
6.3 Results 174
6.3.1 Performance of Hybrids 174
6.3.2 Heterosis 188
6.3.3 Diallel Analysis by Hayman's Method 199
6.3.4 Diallel Analysis using Griffing's Method 214
6.3.5 Genetic Components of Variation 238
6.3.6 Heritability of Traits on Hybrids 244
6.3.7 Relationship between Genetic Distance Based on Microsatellite DNA Markers and Hybrid Performance 248
6.4 Discussion 254

VII GENERAL DISCUSSION AND CONCLUSION 267

REFERENCES 272

APPENDICES 302

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